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Yu. S. Derkich, et al

Army Medical Research Institute of Infectious Diseases
Frederick, Maryland

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US Army Medical Research Institute
of Infectious Diseases
Fort Detrick, Md. 21701

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STUDY OF THE PROTEIN COMPONENTS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS WITH USE OF ELECTROPHORESIS AND ISOELECTRIC FOCUSSING

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(The presence of three protein components with molecular weights of 59,000, 34,000 and 18,000 daltons was determined from mobility in polyacrylamide gel containing sodium dodecysulfate was demonstrated. The method of isoelectric focussing in two modifications (column focussing and focussing on the basis of disk polyacrylamide gel) was used to demonstrate also the presence of three protein-containing components in low-acid, neutral and low-alkaline zones of pH gradient both by ultraviolet adsorption and by radioactive label).

The starting point of our study consisted of data on the structure and protein composition of Group A arboviruses, to which the model studied belongs—namely the virus of venezuelan equine encephalomyelitis (VEEV).

The electron microscope study of the virions of Group A arboviruses enabled us to establish that these consist of an external lipoprotein envelope, a basal membrane, and an inner body containing RNA and protein [3].

At the same time the electrophesis method in polyacrylamide gel was used to demonstrate the presence of two (or three) proteins in various representatives of the Group A arboviruses [4-8].

Proceeding from this, we found it expedient, using a VEEV as a model, to study the protein components of a virus purified by combined agents, basing our study on the data of electrophoresis in agar-polyacrylamide gel, and employing also the method of isoelectric focussing of proteins in two modifi-

cations.

Material and Methods

The VEEV (SPF strain) was passivated in preliminarily trypsinized chicken fibroblasts (monolayer and culturing of cells in [illegible]....) [1].

The infection titer in plaque-type units per 1 ml and the amount of hemagglutinin (in hemagglutinin units per 1 ml) were determined by the commonly used methods, such as were described earlier [1].

Radioactive isotopes. In most of the tests, to determine the radioactive level of the virus we used C^{14}-hydrolysate of chlorella (Radioisotope Center at Leningrad).

Obtaining C^{14}-Marked Virus. A two-day single-layer cell culture of chicken fibroblasts was infected with a multiplicity of 5-10 BPU/cell, and immediately following a triple washing the cells from the virus were placed in an accumulation medium at 5 μc/ml of C^{14}-hydrolysate of chlorella. After 24 hours the yield of virus was collected, and infection hemagglutinating activity and also the level of inclusion of the label were determine. As an accumulation medium we used Earl's solution with 0.5% beef serum; this appeared to give optimal results [2].

Concentration and purity of the virus, and also the means of obtaining individual fractions of the virus, were described in [2].

Method of Electrophoresis in Agar Polyacrylamide Gel. We employed the methods described in [7, 9] with only slight modification.

Preparations of the purified virus (fractions from the cesium chloride gradient with density of 1.25-1.23 g/cm^3) were solubilized in a mixture with the following composition: 1/10 of volume glacial acetic acid; 1% dodecylsulfite of sodium; 1 M of urea; 1% of mercaptoethanol (terminal concentrations are

indicated), incubated for [numeral illegible] hours at 37°C, dialyzed against 0.01 M of phosphate buffer ph 7.2 with 0.1% dodecylsulfite of sodium, 0.5 M of urea and 0.1% of mercaptoethanol. Following this the samples, with protein content of 20 μg, were compressed on disk gels in a volume of 0.2 ml. The composition of the gel mixture was as follows: 10% of acrylamide; 0.5% of N, N'-methylene-bis-acrylamide; 0.1% of sodium dodecylsufite; 0.1 M of sodium phosphate buffer. pH 7.2; 0.03% of N, N, N', N'-tetramethylethylene diamine, and 0.07% of ammonium persulfate. The electrophoresis was conducted in the American-built Polyanalyst apparatus. Further details of the techniques of the electrophoresis, and on the method of computing the molecular weights of the components can be found elsewhere [2].

Study of VEEV proteins was conducted using the method of isoelectric focussing devised by the LKB firm of Sweden [10].

We made an attempt to utilize disk polyacrylamide gel for the isoelectric focussing of the proteins. Two methodological variations were employed: 1) use of carbon electrodes, and 2) use of acid-base locks. The principles of the two variations are shown in Fig. 1.

To destroy the virus, 1 % dodecylsulfite of sodium (final concentration), sludge-80 with ether (1:2), sludge with ether (1:2) were used. The dodecylsulfite was removed by dialysis. Solubility of the proteins was raised and concentration (up to 2.5%) of the ampholyte carriers increased; also by the addition of solubilizing agent agent as urea.

As a stabilizer of pH gradient (range of 3.0-10.0) we used a gel of the following composition: acrylamide (final concentration 5%), bis-acrylamide, one-twentieth part of the concentration of the acrylamide, and ampholyte-

carriers (final concentration 2%). The gel was polymerized photocatalytically. As a catalyst we used a solution of the following composition: 4 ml of riboflavin were added to a volume of 100 ml of water, the solution was filtered, and amphybolite-carrier up to a concentration of 0.5%. As casting forms we used the glass tubes from the "Rezinal" set produced in Hungary.

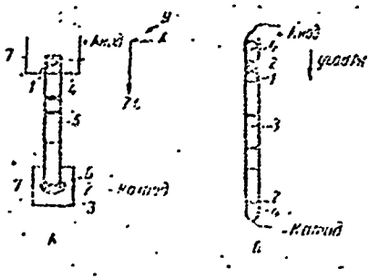

Figure 1. Layout of protein-fractinating apparatus, with use of isoelectric focussing.

A - Modification with acid-base locks; 1 - sample containing virus protein; 2 - .....[illegible] corresponding pH range; 3 - alkali lock; 4 - acid lock; 5 - disk polyacrylamide gel with....[illegible], 2%; 6 - ...[illegible]; 7 - ...[illegible; B - Modification with carbon electrodes: 1 - sample containing protein; 2 - ampholine containing dianaline; 3 - disk gel with ampholine (2%); and 4 - carbon electrodes

The focussing process was conducted at 100 amps for a period of 24 - 28 hours. Into each tube was placed 150-200 μg of protein tagged with C^{14}. The conclusion of the process was controlled by minimal values of current on a milliammeter. The disk gel was prepared with a special cutter to form disks 1 mm in thickness. The fractions were dried on filter paper and placed in scintillation fluid (2,5-diphenyl...., 5 g, 22 n-pnenyl-bis (5-phenyloxasol), 0.3 g, toluene, 1 l) and the radioactivity was recorded (Trocarb, Spectrometer

Packard, USR). The control tube was cut into several equal portions, in order to secure fraction elution of the ampholyte-carriers and to record, over the period of a day, the values of pH with the micromethod (amount of elute up to 1 ml).

For isoelectric focussing [11] a column of 110 ml volumn was used; this was in the form of a vertical tube with internal and external jackets for thermostating, and also upper and lower electrodes. At the bottom is a stopper with tube.

In a solution of the light component were dissolved ampholyte-carriers (pH range 3.9-10.0). The solubilizing agent was urea. For agatation of the solutions in the column was employed an LKB 10200 Ierneks pump.

Saccharose was added to the light component in the amount of 40 mg of tagged purified birus (precursors H^3-uridine, C^{14}-hydrolyzate of chlorella). The virus protines were obtained by destruction with type 80 ether. The actual separation required 72 hours. The stabilizing action of the density gradient and the construction of the column prevented displacement of the separation balance of the substances. In a subsequent study we used a fraction collector with the LKB,7000 cooler (Ulyrarak). As analyzer recording the ultraviolet absorption in the flow, the Unikord LKB 309 was used.

Results. Electrophoresis in gel. Analysis of the proteins of VEEV virus in agar-polyacrylamide gel showed the presence of three distinct peaks of radioactivity (Fig. 2). Proceeding from the relative migration of the protein components, we calculated their molecular weights. As markers we used beef serum albumin, trypsin, ribonuclease, all obtained from the American Koch-Light firm. The method used for calculating the molecular weights has been described above. The molecular weights turned out to be 59,000, 34,000 and 16,000 daltons.

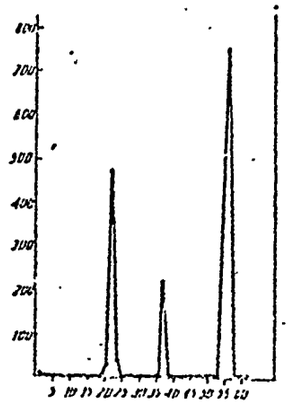

Figure 2. VEEV proteins in 10% polyacrymide gel.

On the y-axis - radioactivity (in imp/min $\cdot 10^{-2}$); on the x-axis- number of the fraction

Isoelectric focussing. A.- Modification in disk polyacrylamide gel. Depending on the method of destruction, we obtained the following results: when sodium dodecylsulfite was used in processing the virus, the proteins, obtaining an anion "coat" were focussed in one zone of the pH gradient (Fig. 3-A). In the case of the use of twine-80 or twine-20 with ether (1:2) as detergents, we obtained three protein components in weak acid, neutral or alkaline zones (Fig. 3B). B. Column isoelectric focussing. In developing the same material with twine-80 with ether visually in the focussing process during the second day we observed three bands of precipitation in weak-acid, neutral and weak-alkaline zones. Upon analysis of the fractions following elusion in the column, there was noted a coincidence of the peaks of ultraviolet absorption and radioactive label; this indicated the presence of three protein components (Fig. 4)

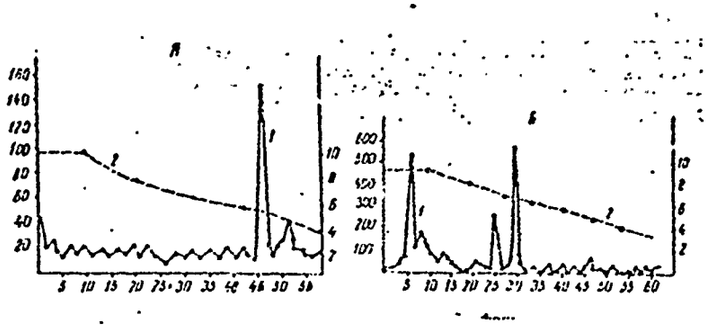

Figure 3. VEEV proteins in conditions of isoelectric focussing (pH, 3.0-10). Focussing for a period of 24 hours. Acid concentration, H_3PO_4; base concentration, 1% NaOH, with carbon electrodes. 200 µg of protein were deposited on the tube. Concentration of ampholine, 2%. Polyacrylamide gel, 5%. On the left, processing of protein with sodium dodecylsulfite; on the right, processing with twine-20 with ether (1:2). 1 - radioactivity; 2 - pH gradient; on the x-axis, fraction number.

Discussion

At the present moment, a certain inconsistency exists in the data published on this subject of proteins in Group A arboviruses. Thus, the method of electrophoresis and polyacrylamide gel has made it possible to show that within the composition of the purified Sindbis virus there are at least two proteins [7], one of which enters into the composition of the membrane, the other into that of the nucleoid. Yin and Lockart [12] have obtained an analogous result, while Horzinck and Mussgay [8] have reported the presence of a third protein component. Friedman [4] has also reported three proteins in the structure of the Semliki Forest virus, two of which form part of the ribonucleoteid of the virus with a sedimentation constant of 140 S, derived from infected cells. Finding two main proteins, and indicating the presence of a minor component in the Chikungunya virus, Igarashi et al. [3] express the suggestion that this differing number of proteins in the Group A arboviruses is explained by structural

peculiarities of the various viruses, or by variation in methodological procedures used in obtaining virus components and by differences in the degree of purification.

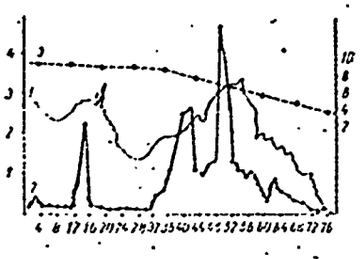

Figure 4. Proteins of the VEEV, as investigated by the method of column isoelectric fokussing.

pH gradient, 3.0-10.9. Duration of focussing, 72 hours. Concentration of amphiline, 1.5%. 1 - registration of infra red absorption. 2 - radioactive C^{14} marker with chlorella hydrolyzate. 3 - pH gradient. On the y-axis, radioactivity (in imp/min...) on the right, pH values. On the x-axis, fraction numbers.

The use of two methods--electrophoresis and polyacrylamide gel and isoelectric focussing of proteins (in two modifications), makes possible a conclusion regarding the composition of high-purity virions of VEEV (Strain SPI) of three protein-containing components. However, making such a conclusion on a clean model, we do not consider as justified its extension to the protein composition of all Group A arboviruses.

B I B L I O G R A P H Y

1. Zhdanov, V. M., Yershov, F. I., Uryvayev, L. V. et al. Vopr. virulog., 1970, No. 1, p. 38.
2. Uryvayev, L. V., Derkach, Yu. S. Yershov, F. I. et al. Biokhimiya (Biochemistry), 1971, No. 1, Vol. 6, p. 92.
3. Igarashi, A. et al, Bikenis J., 1970, Vol. 13, p. 93.
4. Friedman, R. M. J. gen. Virol., 1968, Vol. 2, p. 1076.
5. Hay, A. I., Skehrl, I. Burke, D. C., ibid., 1968, Vol. 3, p. 175.
6. Sreevalsan, T., Allen, P. T. J. Virol., Vol. 2, p. 1038.
7. Summers, D. F., Maizel, I. V., Darnell, J. E. Proc. nat. Acad. Sci. USA, 1965, Vol. 54, p. 505.
8. Horzinek, M., Mussgay, M. J. J. Virol., 1969, Vol. 4, p. 514.
9. Strauss, I., Burge, B. W., Plellerkorn E. R. et al. Virology, 1968, Vol. 37, p. 367.
10. Svensson, H. Acta chem. Scand., 1961, Vol. 15, p. 325.
11. Vesterberg, O., Svensson, H. Ibid., 1966, Vol. 20, p. 820.
12. Yin, F. H., Lockart, R. Z. J. Virol., 1968, Vol. 2, p. 728.

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The presence of three protein components with molecular weights of 50000, 31000 and 18000 daltons determined from mobility in polyacrylamide gel containing sodium dodecylsulfate was demonstrated.

The method of isoelectric focussing in two modifications (column focussing as well as focussing in the basis of disk polyacrylamide gel) was used to demonstrate also the presence of three protein-containing components in low acid, neutral and low alkaline zones of pH gradient both by the ultraviolet adsorption and by radioactive label.